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13. ABSTRACT (Maximum 200 Words)

The human steroid 5-alpha reductase type I gene (SRD5A1) was examined for genetic changes in four ethnic groups: African-Americans, Latinos, Caucasians, and Japanese-Americans. SRD5A1 was examined because its gene product encodes an enzyme that can reduce androgens, which are known to be involved in modulating prostate gland growth, maintenance, and differentiation. In an attempt to identify common single nucleotide polymorphisms (SNPs) and establish allele frequencies, leukocyte genomic DNA from 118 men (half normal controls and half diagnosed with prostate cancer) was examined for SRD5A1 genetic variants. The open reading frame (5' and 3' untranslated regions (UTR), codons, and some intronic sequence) was sequenced, plus over one thousand base pairs upstream of the gene. Twenty-eight DNA changes were discovered along the length of the gene. Most SNPs were transitions (88%) but there was one single base deletion in the putative promoter (which was confirmed using restriction fragment length polymorphism). The six codon SNPs were silent; however, there was linkage disequilibrium between the two SNPs in exon 2, forming a haplotype. With the genetic screen of SRD5A1 variants completed, functional testing of promoter and 3'UTR variants are currently being assessed in cell lines for changes in promoter activity, RNA steady-state and, half-life.

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Introduction:

For over sixty years, the link between androgens and prostate cancer has been established (Huggins, C.B., Hodges, C.V. 1941). Early studies demonstrated the effect that castration had on reducing or eliminating prostate carcinoma, while injections of testosterone (a steroid hormone) had the opposite effect. Later work demonstrated that steroid hormones bind to receptors and regulate nuclear gene regulation, which alters cell and tissue phenotypes (Lefkowitz, R.J., et al. 1970; Attramadal, A., et al. 1976) Thus, enzymes that control the androgen metabolic flux are of interest in prostate cancer predisposition studies.

Publications have supported the hypothesis that the development of prostate cancer is androgen dependent and that heritable factors account for about 42% of the risk (Gann et al. 1995; Lichtenstein, et al. 2001). Although most sporadic cancers are caused by environmental factors and de novo mutations, the relatively large genetic contribution in prostate cancer demonstrates how wide are the gaps in our knowledge and understanding of cancer genetics (Lictenstein, P., et al. 2000). Therefore, an examination of the different enzymes that catalyze the anabolism of cholesterol into dihydrotestosterone (DHT) should be useful at elucidating how increased metabolic flux through these pathways impact prostate growth, and therefore, the likelihood of being afflicted with tumors (Davies, et al. 1991; George, et al. 1991; Wilson, et al. 1975). There are two known steroid 5-alpha reductases in humans, types I and II. The two genes lie on different chromosomes, have similar gene structure, and encode steroid 5-alpha reductases, but show different tissue expression. The type II reductase has been studied in relation to prostate cancer and certain SNPs may play a role in some human populations.

The steroid 5-α reductase type II enzyme (E.C. 1.3.99.5), which catalyzes the reduction of testosterone (and other steroids) into the physiologically more active dihydrotestosterone (DHT) was shown to have at least ten single amino acid substitutions and three double mutations, all of which occur in normal, healthy males (Makridakis, et al. 2000). The kinetic properties of these mutant enzymes have been measured, with some showing larger or smaller values than the wild-type enzyme (Makridakis, N., et al. 1999; Makridakis, N., di Salle, E., Reichardt, J.K.V. 2000). One mutation, which changes an alanine at amino acid 49 into a threonine, boosts African-American men's chances of developing prostate cancer 7.2 fold, while the same mutation imparted Latinos with 3.2 times the likelihood of developing the disease (Makridakis, et al. 2000). This poses the question: Does the related type I reductase possess variants in human populations which can mutate the enzyme thus predisposing/protecting those men to prostate cancer or benign prostatic hyperplasia (BPH)?

Specific Aims

The SRD5A1 gene encodes the steroid 5-α-reductase enzyme type I which reduces testosterone into a physiologically more active chemical form, dihydrotestosterone (DHT). I hypothesize that the human steroid 5-α reductase type I enzyme plays a role in prostate cancer development, and that gain-of-function single nucleotide polymorphisms (SNPs) in the SRD5A1 gene can increase androgen metabolic

flux to the prostate, increasing its size, and thus predisposing to prostate cancer over decades of life.

***Specific Aim #1** of this study involved uncovering SRD5A1's genetic variation in African-American, Latino, Caucasian, and Japanese-American populations.*

Results:

- Figure 1 shows a schematic diagram of the human SRD5A1 gene. Uncovered SNPs are numbered with lines. Note that numbering is based on the cDNA (accession#NM_001047), while putative promoter SNPs are number relative to the beginning of the mRNA.

- Table 1 lists the SNPs, their locations, genotypes, allele frequencies, and numbers of chromosomes sequenced from the population.

- One hundred eighteen genomic leukocyte DNA samples from men comprising 4 ethnicities were DNA sequenced along its open reading frame, and over one thousand base pairs upstream of the SRD5A1 gene. Half of the samples are from men who have been diagnosed with prostate cancer, and the other half from normal men. All samples were blinded to prevent ascertainment bias.

- Twenty-eight different single nucleotide polymorphisms were discovered in the above population.

- Thirteen SNPs in the putative promoter region, six in the codons, three intronic, and three in the 3' untranslated region (3'UTR).

- Eighty-eight percent of SNPs were transitions, but at SNP –382, some men were heterozygous for a single base deletion (delA). The deletion was confirmed using restriction fragment length polymorphism analysis.

- The two exon 2 SNPs (436 and 475) were found to be in strong linkage disequilibrium, thus forming a haplotype.

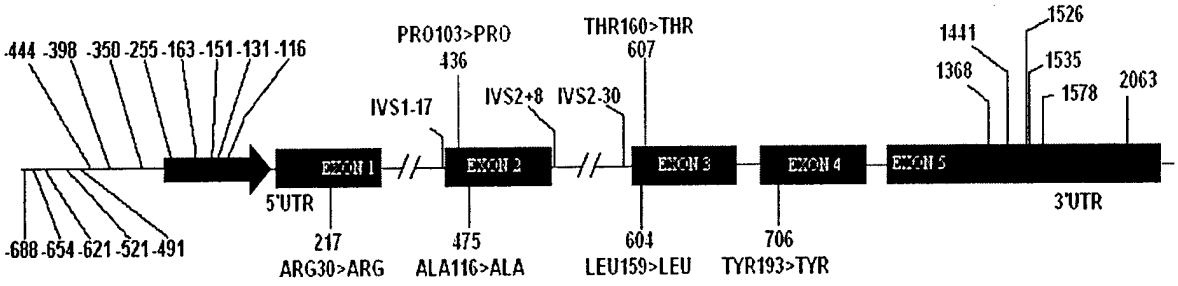

Figure 1. Schematic of the human steroid 5-alpha reductase type I gene (SRD5A1). Twenty-eight single nucleotide polymorphisms (SNPs) are indicated with lines and numbering. Amino acids are shown for SNPs in the coding region.

Table 1. Human SRD5A1 SNP locations and frequencies.

Gene Region	SNP	Genotypes	Observed Frequencies (Observed/Total)	Allele	Allele Frequency	Number of Chromosomes Sequenced
PROMOTER	-688G>A	G/G	46/85	G	0.72	170
		G/A	30/85	A	0.28	
		A/A	9/85			
	-654G>A	G/G	28/85	G	0.51	170
		G/A	30/85	A	0.49	
		A/A	27/85			
	-621T>G	T/T	31/85	T	0.55	170
		T/G	31/85	G	0.45	
		G/G	23/85			
	-521C>T	C/C	48/87	C	0.72	174
		C/T	30/87	T	0.28	
		T/T	9/87			
	-491A>G	G/G	0/86	G	0.02	172
		G/A	4/86	A	0.98	
		A/A	82/86			
	-444G>T	G/G	85/86	G	0.99	172
		G/T	1/86	T	0.01	
		T/T	0/86			
	-398G>A	G/G	82/85	G	0.98	170
		G/A	2/85	A	0.02	
		A/A	1/85			
	-350C>T	C/C	51/86	C	0.77	172
		C/T	31/86	T	0.23	
		T/T	4/86			
	-255delA	A/A	76/87	A	0.94	174
		A/del	11/87	del	0.06	
		del/del	0/87			
	-163A>G	A/A	86/87	A	0.99	174
		A/G	0/87	G	0.01	
		G/G	1/87			
	-151C>G	G/G	0/97	G	0.02	194
		G/C	4/97	C	0.98	
		C/C	93/97			
	-131G>A	G/G	82/87	G	0.97	174

		G/A	4/87	A	0.03	
		A/A	1/87			
	-116C>T	C/C	48/85	C	0.85	170
		C/T	24/85	T	0.15	
		T/T	13/85			
EXON 1	217G>C	G/G	31/101	G	0.51	202
		G/C	41/101	C	0.49	
		C/C	29/101			
INTRON 1	IVS1-17G>A	G/G	91/98	G	0.96	196
		G/A	7/98	A	0.04	
		A/A	0/98			
EXON 2	436A>G	G/G	4/99	G	0.27	198
		G/A	46/99	A	0.73	
		A/A	49/99			
	475G>A	G/G	56/99	G	0.76	198
		G/A	39/99	A	0.24	
		A/A	4/99			
INTRON 2	INV2+8T>C	T/T	95/98	T	0.98	196
		T/C	3/98	C	0.02	
		C/C	0/98			
	IVS2-30C>T	T/T	1/100	T	0.05	200
		T/C	8/100	C	0.95	
		C/C	91/100			
EXON 3	604A>G	G/G	0/100	G	0.01	200
		G/A	2/100	A	0.99	
		A/A	98/100			
	607G>A	G/G	57/100	G	0.76	200
		G/A	37/100	A	0.25	
		A/A	6/100			
EXON 4	706C>T	T/T	0/103	T	0.02	206
		T/C	5/103	C	0.98	
		C/C	98/103			
3'UTR	1368C>T	T/T	23/100	T	0.43	200
		T/C	40/100	C	0.57	
		C/C	37/100			
	1441G>A	G/G	88/95	G	0.96	190
		G/A	6/95	A	0.04	
		A/A	1/95			
	1526A>G	G/G	3/99	G	0.07	198
		G/A	8/99	A	0.93	
		A/A	88/99			

	1535G>A	G/G	94/100	G	0.97	200
		G/A	6/100	A	0.03	
		A/A	0/100			
	1758C>T	T/T	1/99	T	0.04	198
		T/C	6/99	C	0.96	
		C/C	92/99			
	2063T>C	T/T	99/103	T	0.98	206
		T/C	4/103	C	0.02	
		C/C	0/103			

Specific Aim #2 *entails identifying how SNPs alter steroid 5-α reductase type I function in biochemical and kinetic assays.*

Update:

Specific Aim #2 will biochemically characterize SNPs in the putative promoter/enhancer region, and the 3'-UTR. SNPs in the promoter can change the amount of transcript made (Hoogendoorn, B., et al. 2004), or alter RNA steady-state or half-life (di Paolo, et al. 2002). Changing the amount of SRD5A1's mRNA could alter the total amount of enzyme available for catalysis, and could influence the androgen metabolic pathway, thus modulating prostate gland growth.

The most common method of evaluating SNP functionality is to clone the region containing the SNP in front of (for a promoter or enhancer SNP), or behind (in the case of 3'-UTR SNPs) a reporter gene like firefly luciferase and transfecting the vector into mammalian cell lines. The cells are lysed, and luciferase specific activity is measured. If luciferase specific activity changes with a SNP as compared to the wild-type, then this indicates that the SNP is important, and should be genotyped in a larger population for a role in prostate cancer. While it is not possible to predict the number of SNPs that will affect luciferase specific activity, a recent large-scale effort examining SNPs in 170 human promoters found that roughly one third of promoter SNPs altered luciferase specific activity compared to the control (Hoogendoorn, B., et al 2003). If this same rate holds true for the SRD5A1 gene, then I would expect 4 of the 13 SNPs to functionally alter SRD5A1 expression. I expect Specific Aim #2 to take approximately one year to complete.

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